The synthesis and photophysical properties of novel indolizine-based fluorophores

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In the quest to develop small fluorescent molecules for bioimaging applications, this work focused on a deeper understanding of π-expanded indolizines; the improvement of existing pathways for the synthesis of known cores; the development of novel strategies for new ones; the rationalization of the relationship between substituents and photophysical properties; and, finally, the investigation of promising candidates for bioimaging applications.

Around this topic, three articles and a review were published, all of which are included in this dissertation.

The first project I worked on led to the publication *"The Kröhnke synthesis of benzo[a]indolizines revisited: towards small, red light emitters"*, in which I described the improvement of the synthetic methodology and the broadening of the scope for obtaining **benzo[*a*]indolizines**, a class of π-expanded indolizines first described by Kröhnke in the 70s, and the rationalization of their photophysical properties in relation to the different substituents introduced.
Kröhnke described the synthesis of benzo[*a*]indolizines starting from pyridinium salts and 1-chloro-2,4,6-trinitrobenzene in two steps, using first an inorganic base, K_2CO_3 in $CHCl_3$, followed by an organic base, piperidine, in DMSO. This methodology led to a limited number of products in relatively low yield.
The first change I implemented over the proposed path was the substitution of the highly explosive 1-chloro-2,4,6-trinitrobenzene with the less hazardous 4-chloro-3,5-dinitrobenzene derivatives, yielding three products in 20–30% yield. The second was the identification of a more suitable combination of base and solvent to avoid the isolation step of the intermediate. After a series of attempts using different bases and solvents to enhance the yields, I was able to reduce the number of synthetic operations, increase the yields, and accelerate the reactivity of the pyridinium salt and the 4-chloro-3,5-dinitrobenzene derivatives by employing Cs_2CO_3 and sulfolane. This led to the isolation of eleven different products in yields ranging from 5 to 80%. Further functionalization afforded two additional derivatives.
Once a more efficient synthetic pathway was established, attention was turned to the photophysical investigation. The analysis of the photophysical properties based on the different substituents and their positions allowed for a rationalization of the structure-photophysics relationship of this class of fluorophores (fig 1).

In the second project I followed the same general approach and worked on optimizing the methodology for the synthesis of **2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylates**, first proposed by Kakehi in the 90s, and on investigating their photophysical properties. This work led to the following publication: *"Strongly fluorescent indolizine-based coumarin analogs".*
Starting from pyridinium salts and diethyl 2-(ethoxymethylene)malonate Kakehi obtained products in one-pot reaction after one week using K_2CO_3 as base in EtOH. I instead switched the base to Cs_2CO_3 and used a diverse array of pyridinium salts, preparing 8 products in 5-50% yield. Further functionalization was performed, and five additional structures were obtained.
With a plethora of 2-oxo-2H-pyrano[2,3-*b*]indolizine-3-carboxylates available, the photophysical investigation and rationalization of the fluorescent properties was carried out (representatives

displayed in fig. 1). Among those, the styryl dye was selected for H9c2 cell line staining and demonstrated, after permeabilization with digitonin, selective localization in the nuclei.

The last project extended beyond the improvement and expansion of previous works and resulted in the discovery a completely new class of fluorophores which culminated with recently published article: *The Hybrid of Indolizine and Merocyanine – A New Class of Organelle-specific Dyes.*
In this work, starting from indolizin-ol intermediates and 2,3,5,6-tetrafluoro-4-hydroxybenzaldehyde in xylene, I obtained six hybrids polymethine dyes which I called **indolizine-merocyanines (IndMers)**, in a one-pot procedure with yields ranging from 26-90%. The developed methodology was also tested with different aldehydes of similar characteristics, generating two additional heretofore structures: a V-shaped xanthene dye and a cyanine type dye.
A comprehensive photophysical investigation was carried out on the obtained structures (representatives are shown in fig. 1) providing a broad understanding of their structure-photophysics relationship. In addition, for some selected structures, photostability and solubility in aqueous media were investigated.
Among those compounds one showed emission in the NIR region, solubility in aqueous media, high cell permeability in HT1080 fibrosarcoma and MRC5 normal human lung fibroblast cells, and context dependent accumulation in mitochondria and RNA rich nucleoli when compared to MitoTracker™ Green FM and SYTO RNASelect™ respectively suggesting our success in developing a new dye for bioimaging.

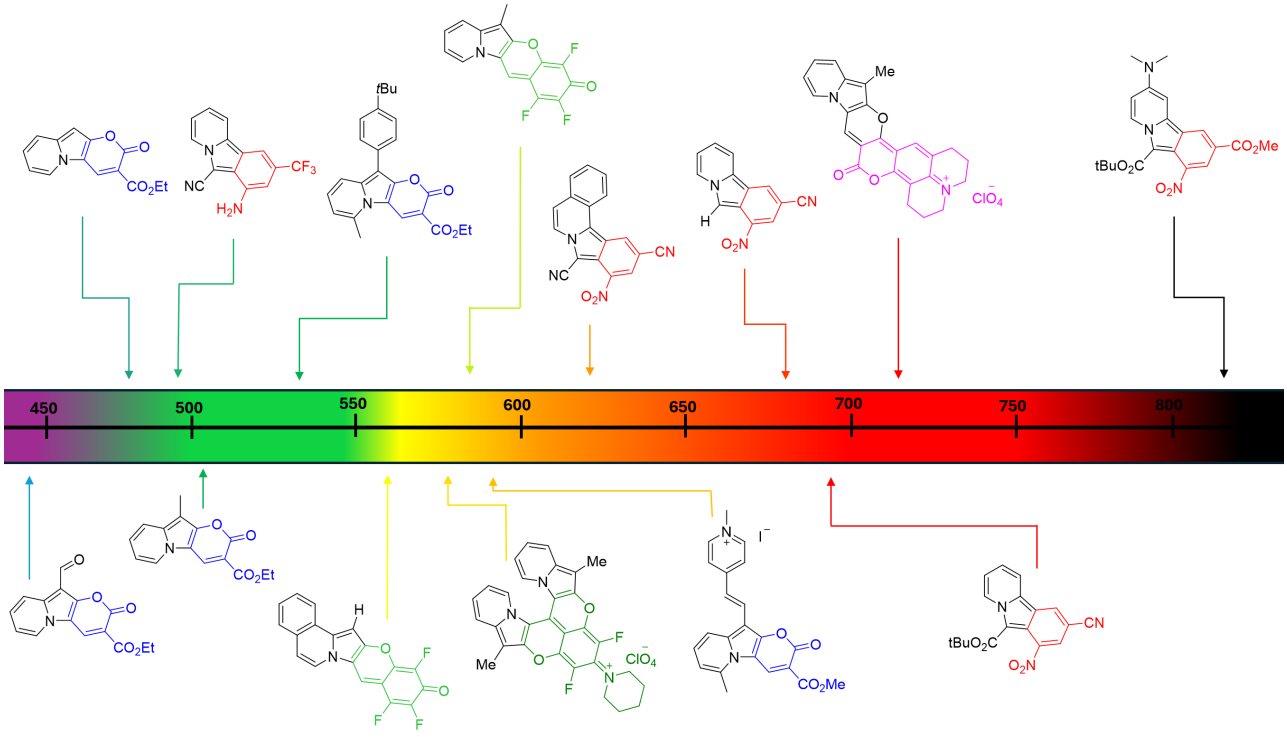

Figure 1: *Structure and visual comparison of the fluorescence emission of some representatives of benzo[a]indolizines, pyrano[b]indolizines and IndMers in DCM.*